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ETHANOLIC EXTRACT OF *Phoenix dactylifera* L. PREVENTS LEAD INDUCED HEMATOTOXICITY IN RATS

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ABSTRACT

Lead is a heavy metal that has been known for its adverse effects on many body organs and systems and thus their functions. In this study, the toxic effect of lead on blood was investigated, and ethanolic extract of *Phoenix dactylifera* L. (EPD) (a well known nutritious, antioxidant, and medicinal fruit) was administered orally to prevent lead's toxicity. Forty adult male Wister rats, randomly divided into four groups (n = 10), were used for this study Group B and Group D were given 200 g of EPD/Kg Body Weight/Day (orally) and 1% sodium acetate and Lead acetate respectively, while group A (control) and group C were given sodium acetate and lead acetate respectively. All treatments were for eight weeks. The animals were weighed and then sacrificed twenty-four hours after the last treatment. Hematocrit, red and white blood cell counts (RBC and WBC), differential count, hemoglobin concentration and other hematological parameters were determined. The data obtained were compared using t-test. The results showed that lead caused a significant decrease in hematocrit, RBC, WBC, hemoglobin concentration, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and lymphocyte and monocyte count; and significant increase in neutrophil count. These were, however, prevented in the EPD treated groups. It is therefore concluded that oral admistration of EPD promotes blood's health and annuls lead induced hematotoxicity.

KEY WORDS: *Phoenix dactylifera*, lead, blood, anemia, date palm

INTRODUCTION

Lead is one of the first metals to be smelted and used (Aicheson, 1960). It probably was first mined in Turkey around 3500 BC (Alan, 2010). Its density, workability and corrosion resistance were among the metals attractions (Flora, 2008). Reasonably, lead poisoning [plumbism or painter's Colic (Kosnett, 2006)] is one of the oldest known environmental hazards (Flora, 2008). Also, no level of lead in the body below which no harm can occur has been discovered (Merrill et al., 2007). Worst still, the levels of lead found in most people today are of magnitudes greater than those of pre-industrial times (Merrill et al., 2007; Patrick 2006). In fact, humans get exposed to lead through various media: air, water, soil, food and consumer products (Gärtner, 1997; Gidlow, 2004; Thompson, 2006).

Lead interferes with a variety of body processes and is toxic to the body systems including the cardiovascular, reproductive, haematopoietic, gastrointestinal, and nervous systems (Kosnett, 2006), renal functions (Patocka and Cerný, 2003), release of glutamate and learning (Xu et al., 2006). It affects the hematological system [even at concentrations below 10µg/dl (ATSDR, 2005)] by inhibiting the activities of several enzymes [particularly Amino Levulinic Acid Dehydratase (ALAD)] involved in heme biosynthesis and shortening of erythrocyte life span (Sakai et al., 1999) as well as inducing inappropriate production of erythropoietin leading to inadequate maturation of red cell progenitors and affecting the introduction of Fe^{2+} into protoporphyrin IX (Taketani *et al*., 2001). Even though manifestations of Pb poisoning in humans are non-specific, they are always accompanied by oxidation (Hande *et al*., 2004).

On the other hand date palm (*Phoenix dactylifera*) fruits, an important component of diet in the arid and semiarid regions of the world (Biglari *et al*., 2008), are widely used in traditional medicine for the treatment of various

disorders e.g. memory disturbances, fever, inflammation, paralysis, loss of consciousness, nervous disorders (Nadkarni, 2006), and as a detersive and astringent in intestinal troubles. It is also used in the treatment for sore throat, colds, bronchial asthma, to relieve fever, cystitis, gonorrhea, edema, liver and abdominal troubles and to counteract alcohol intoxication (Barh and Mazumdar, 2008). It possesses anticancer, antimutagenic, antihyperlipidemic, nephroprotective, and *in vivo* antiviral activities, and the ability to increase the concentration of testosterone, follicle stimulating hormone and luteininzing hormone (Bahmanpour et al., 2006). Many researchers have also documented the antioxidant property of *Phoenix dactylifera* (Mohamed and Al-Okbi, 2004; Allaith and Abdul, 2005; Al-Qarawi *et al*., 2008).

Since humans are inseparable from their environment and almost entirely not free from exposure to Lead (most especially that lead has almost always found an important position in many industries), it is very necessary to figure out ways by which our body can be made to still maintain homeostasis (basis of good health) even on exposure to relatively high level of lead exposure. This research work, therefore, aims at knowing whether or not oral administration of extract of *Phoenix dactylifera* (EPD) prevents lead induced hemato-toxicity base on the knowledge that EPD has nutritious, medicinal, and antioxidant components.

MATERIALS AND METHODS

Forty adult male Wister rats [average Body Weight (BW) 186.70 ± 0.511 g] obtained from the animal house section of Faculty of Pharmaceutical Science, Ahmadu Bello University, Zaria, Nigeria, were used for this study. The animals were kept in the animal house section of Human Physiology Department, Ahmadu Bello University, Zaria, Nigeria, and allowed to acclimatize over a period of ten days.

PLANT MATERIALS

The fruits of *Phoenix dactylifera* were bought from a local market in Zaria, Kaduna State, Nigeria and authenticated at the Department of Biological Science, Ahmadu Bello University, Zaria, Nigeria.

PREPARATION OF ETHANOLIC EXTRACT OF *PHOENIX DACTYLIFERA* (EPD)

The extract of the fruits was prepared in the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria. The extract was prepared by removing the shells of the fruit and the fruit grounded into fine powder. The fine powder was left to dry under shade, it was soaked in ethanol (inside a separating funnel plugged with cotton wool) for 24 hours. It was drained and rewashed with more ethanol. The filtrate was poured in an evaporating dish and placed on a water bath at 80 ^{o}C, and reduced pressure (70% atmospheric pressure) to remove the solvent. After all the solvent had evaporated, the extract was scrapped and stored in a dry glass container at a temperature of 0 – 4 ^{o}C. Fresh solution of the extract was prepared in distilled water just before use to maintain its potency.

ANIMAL TREATMENT

The forty rats were randomly grouped into four (Group A, B, C and D, n = 10). Rats in group A served as the control and were neither exposed to lead nor treated with EPD, but they were allowed to drink 1% sodium acetate ad libitum. Group B rats were allowed to drink 1% sodium acetate ad libitum and also administered 200 mg of EPD/Kg BW/Day. Group C and D were allowed to drink 1 % lead acetate ad libitum, while group D rats were in addition administered 200 mg of EPD/Kg BW/Day. All treatments were for eight weeks.

ANIMAL SACRIFICE AND COLLECTION OF SAMPLES

Twenty-four hours after the last treatment, each animal was weighed and then sacrificed by cervical dislocation and blood samples were collected via cardiac puncture. Blood sample obtained from each rat was immediately transferred into EDTA bottle and mixed thoroughly although gently.

COLLECTION OF DATA AND STATISTICAL ANALYSIS

PCV was determined using plain capillary tubes filled with anti-coagulated blood and spinning at 5.1 x g for 20 min. RBC and WBC were determined using Improved Neubauer counting chamber and following the procedure documented by Chessbrough (1976). Field's stain A and Field's stain B were used for the Differential WBC.
The control and "Test groups" were compared using t-test. The significant level was set to P value < 0.05.

RESULTS

The following results were obtained and are presented as mean ± SEM and level of significance is taken at "p value < 0.05" (*), "p value < 0.001" (**) and/or "p value < 0.0001" (***).

WEIGHT INCREASE (G)

Comparing their final and initial weight showed that there was significant weight gain (*P*-value < 0.05) is in all the groups over the 10 weeks of the research. There was, however, no significant difference (*P*-value > 0.05) in weight gain of NA+EPD$_G$, LA+EPD$_G$ and Control, while weight increase in LA$_G$ had a significantly lower value when compared to the Control (Table 1).

PACKED CELL VOLUME (PCV, %), RED BLOOD CELL COUNT (RBC, CELLS/mm^3), AND WHITE BLOOD CELL COUNT (WBC, CELLS/mm^3), HEMOGLOBIN CONCENTRATION (Hb, G/DL), MEAN CORPUSCULAR VOLUME (MCV, FL), MEAN CORPUSCULAR HEMOGLOBIN (MCH, PG), AND MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (MCHC, G/DL)

The PCV and RBC of NA+EPD$_G$ and LA+EPD$_G$ were significantly (*P*-value < 0.05, and *P*-value < 0.001 respectively) higher than that of the control. However, PCV and RBC of LA$_G$ was found to be significantly (*P*-value < 0.05) lower than that of the control. Similar trends were noted for Hb, MCH, and MCHC. While WBC and MCHC of LA$_G$ were found to be significantly (*P*-value < 0.01) lower than that of the control; but LA+EPD$_G$ and NA+EPD$_G$ showed no significant (*P*-value > 0.05) difference from the Control (Table 2).

DIFFERENTIAL COUNT

Differential count showed that LA$_G$, unlike NA+EPD$_G$ and LA+EPD$_G$, has significantly (*P*-value < 0.05) lower percentage of Lymphocyte and Monocyte, but significantly (*P*-value < 0.05) higher percentage of Neutrophil. Percentages of Eosinophil and Basophil of NA+EPD and LA+EPD were however not different from that of the Control (Table 3).

DISCUSSION

The results of this study show that chronic exposure to Pb significantly (*P*-value < 0.05) reduces weight gain (Table 1), this is in support of the findings of Suzan and Ghayasuddin (1999) and can be linked to the less efficient metabolic processes associated with Pb toxicity (Struzyńska et al., 1997), that is the anti-metabolic effect of Lead (Biswas and Ghosh, 2004). Administration of 200 mg of EPD/Kg BW/Day, however, annuls this Pb's adverse effect on weight gain; it in fact had a significant (*P*-value < 0.05) enhancing effect on the final BW of group B and group D. This may be partly due to the fatty acid composition of EPD (National Institute for Health and Welfare, 2009); and mainly due to its relatively high caloric value (284 Kcal/100g) (National Institute for Health and Welfare, 2009) and the presence of health-protective antioxidants such as melatonin, vitamin E, and ascorbic acid in EPD (Al-Qarawi *et al.*, 2008) despite its relatively low protein content (6% by weight) (National Institute for Health and Welfare, 2009). These can similarly explain the significant (*P*-value < 0.05) decrease in PCV noticed in animals exposed to Pb (LA$_G$) and the non significant (*P*-value > 0.05) decrease in PCV noticed in animals treated with EPD alongside Pb exposure (LA+EPD$_G$) (Table 2). This finding that Pb reduces PCV is in support of the findings of Anetor *et al.*, (2002) that some indices of erythropoietic activity such as Hb, PCV and MCHC are significantly decreased in workers exposed to lead. It however opposes the findings of Franson et al., (1983) that PCV is not affected in birds by 50 ppm of Pb in their feed; and that of Arvind and Chopra, (2003) that PCV is not affected in calves by 100 ppm Pb in the diet. It is, however, possible that the findings of Franson *et al*., (1983) and Arvind and Chopra, (2003) are due to the low concentration of Pb (</= 100 ppm) administered as well as the relatively short period of administration.

The significantly (*P*-value < 0.05) high RBC in both NA+EPD$_G$ and LA+EPD$_G$, and the significantly (*P*-value < 0.05) low RBC in LA$_G$ compared to the control [Table 2] support the findings of James, (2007) and Salawu et al., (2010) that lead induces anemia especially when the exposure is chronic; and the findings of Abuharfeil et al., (1999) that *Phoenix dactylifera* promotes RBC by preventing hemolysis. These observations could be accounted for by the ability of Pb to interfere with energy metabolism of erythrocytes (Irena and Alina, 2003) which is one of the determinants of ATP concentration in erythrocytes and thus erythrocytes' life span and RBC. This reduction in RBC could still be linked to the findings of Bernard et al., (1972) that acute lead (Pb) toxicity in mice produced transient

erythroid hypoplasia and impaired utilization of RBC ^{59}Fe for heme synthesis. The administered EPD would therefore be responsible for the prevention of these lowering effects of Pb on RBC (and even the enhancement of RBC) in LA+EPD $_{G\backslash}$ probably by preventing its adverse effects on energy metabolism of erythrocytes and on the utilization of RBC ^{59}Fe for heme synthesis. This becomes more evident by the significantly high (*P*-value < 0.05) RBC found in NA+EPD $_G$ as well (Table 2).

The significantly (*P*-value < 0.0001) lower WBC in LA $_G$, and the non-significant (*P*-value > 0.05) difference in the WBC of both NA+EPD $_G$ and LA+EPD $_G$ with respect to the control, further establishes that lead adversely affects blood and blood cells. This observation is in line with the previous publications of Nedjet *et al*., (2009) and Karmaus *et al*., (2005) that lead exposure is associated with a reduction in WBC. It also lends supports to scientific claims that lead exposure may lower body's immunity (Grasman and Scanlon, 1995; Rabinowitz *et al*., 1990), more so that white blood cells are important part of the immune system (Karmaus *et al*., 2005). Therefore, EPD must have somehow prevented Pb's adverse effects on WBC, such that there was no significant difference in WBC of control and that of LA+EPD $_G$. This could most reasonably be linked to the detoxification as well as hepatoprotective effect of EPD (Al-Quarawi *et al*., 2005).

In the present study, just as documented by Sembulingam and Sembuligam (2006) that Pb (like some other chemicals and drugs) causes netrophilia, Pb caused a significant (*P*-value < 0.05) increase in Neutrophil count but significantly (*P*-value < 0.05) decreased Lymphocyte and Monocyte counts (Table 3). This is as well in support of the findings of Di Lorenzo et al., (2006) that mean absolute neutrophil count was significantly higher in workers exposed to Pb with respect to workers not exposed to Pb. In addition Neutrophils and lymphocytes were noticed to vary in opposite direction, which is as well in support of the documentation of Sembulingam and Sembuligam, (2006). However, EPD was able to prevent Pb from affecting not only WBC but differential count as well.
Conclusively, lead causes significant decrease in hematocrit, RBC, WBC, hemoglobin concentration, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and lymphocyte and monocyte count; and significant increase in neutrophil count. However, oral administration of EPD prevents these lead's adverse effects (in rats). It is therefore reasonable to conclud that oral admistration of EPD promotes blood's health and annuls lead induced hematotoxicity.

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Table 1: Weight Increase (g) after the 10 Weeks of Research

	Control	NA+EPD$_G$	LA$_G$	LA+EPD$_G$
Weight before sacrifice (g)	216.17 ± 3.13	206.00 ± 3.61*	196.33 ± 3.95**	204.5 ± 4.74 *
Initial weight (g)	187.33 ± 6.71	189.33 ± 7.50	187.33 ± 7.00	182.67 ± 8.01
Weight increase (g)	28.83 ± 7.47	16.67 ± 5.96	9.00 ± 5.54*	21.83 ± 3.36

* "*P*-value < 0.05"

Table 2: Packed Cell Volume (PCV, %), Red Blood Cell Count (RBC, cells/mm^3), and White Blood Cell Count (WBC, cells/mm^3), Hemoglobin concentration (Hb, g/dl), Mean Corpuscular Volume (MCV, fl), Mean Corpuscular Hemoglobin (MCH, pg), and Mean Corpuscular Hemoglobin Concentration (MCHC, g/dl)

	Control	NA+EPD $_G$	LA $_G$	LA+EPD $_G$
PCV (%)	22.200 ± 0.467	43.300 ± 0.790 **	20.200±0.512*	33.900±1.456 *
RBC (cells/mm^3)	2.545 ± 0.0762	4.930 ± 0.070 **	1.990±0.041*	4.610±0.208 *
WBC (cells/mm^3)	2533.33 ± 29.51	2511.67 ± 63.58	2161.67 ± 32.29 ***	2550.00 ± 113.1
Hb (g/dl)	7.110 ± 0.076	14.270 ± 0.226**	6.540 ± 0.105*	14.710 ± 0.154 **
MCV (fl)	84.100 ± 0.605	86.300 ± 0.423*	83.000 ± 0.577	85.500 ± 0.582
MCH (pg)	30.400 ± 0.542	34.100 ± 0.525*	27.100 ± 0.277*	32.00 ± 0.526*
MCHC (g/dl)	33.300 ± 0.597	35.400 ± 0.476	30.600 ± 0.542*	33.000 ± 0.394

* "*P*-value < 0.05", ** "*P*-value < 0.001", *** "*P*-value < 0.0001"

Table 3: Comparison of Percentage of White Blood Cells that is Neutrophil, Lymphocyte, Monocyte, Eosinophil and Basophil Sperm Motility across the three Groups

	Control	NA+EPD $_G$	LA $_G$	LA+EPD $_G$
Neutrophils (%)	57.83±0.872	56.00±0.894	61.83±0.872*	58.67±1.05
Lymphocytes (%)	31.83±0.872	32.5±0.428	28.83±0.601*	30.83±0.401
Monocyte (%)	6.33±0.667	7.33±0.558	4.667±0.422*	6.00±0.816
Eosinophil (%)	3.167±0.543	2.833±0.307	3.5±0.428	3.50±0.224
Basophil (%)	0.833±0.654	1.333±0.211	1.167±0.477	1.00±0.365

* "*P*-value < 0.05"

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